**REMARKS/ARGUMENTS**

In the present amendment, claims 47-49, 51-56, 60-62, and 64-69 are pending in the current application. Claims 1-43 and 70-77 are withdrawn from consideration as being drawn to a non-elected invention. Claims 47, 56, 60, and 69 are amended herein. The specification has been amended to correct minor clerical errors in paragraphs 42, 79, 88, 112, 196, 220, and 340 of the application. The correct structure for formula III in paragraph 42 appears on page 9 of the application as originally filed; paragraph 42 has been amended accordingly. No new matter has been added by way of the instant amendments to the specification. Support is found throughout the specification (*see*, *e.g.*, Figs. 5A, 5B, 8, and 20; Examples 1 and 3; paragraphs 204 and 227). The citations to the specification included throughout this response are to the paragraph numbers of the published application (US 2004/0175767).

In the present amendment to the claims, claims 47 and 60 are amended to replace Formula I with Formulae II-VIII. Claims 56 and 69 are amended to correct the number of hydrogen atoms bound to a carbon atom in Formula III. Support for the amendments can be found throughout the specification as filed, including paragraphs 203-204 and the original claims. Accordingly, no new matter has been added by way of the amendments to the claims.

Entry of these amendments is respectfully requested. The rejections in the Office Action of January 22, 2007 are addressed individually below.

## I. Rejection Under 35 U.S.C. § 112, First Paragraph, Enablement

Claims 47-49, 51-56, 60-62, and 64-69 were rejected under 35 U.S.C. § 112, first paragraph, for allegedly failing to comply with the enablement requirement. The Office Action opines that claims 47 and 60 lack enablement because the specification does not demonstrate "results that indicate a direct connection between pigmentation and an alteration in late endosomal/lysosomal trafficking," and because the specification does not describe a way to assay endosomal/lysosomal trafficking. The Office Action also opines that claims 47 and 60 lack enablement because "the skilled artisan would be required to determine the 'effective amount' of each claimed compound ... with no direction or guidance from the instant disclosure." Applicants respectfully traverse this rejection.

The specification clearly describes and provides experimental results supporting the link between pigmentation and effects on late endosomal/lysosomal trafficking. This disclosure provides sufficient teaching to allow one of ordinary skill in the art to identify compounds that affect late endosomal/lysosomal trafficking and also impact pigmentation, and to assess effective amounts of such compounds without undue experimentation.

For example, the specification explains that tyrosinase is key to melanogenesis (¶ 8), and describes in detail the importance of tyrosinase localization for proper melanogenesis (¶¶ 21-22, 77-80). The specification further explains that compounds that affect late endosomal/lysosomal trafficking impact the localization of tyrosinase and other proteins necessary for melanogenesis, and therefore impact melanogenesis (¶¶ 30, 78, 80). Compounds that affect late endosomal/lysosomal trafficking and therefore affect melanogenesis may impact trafficking of molecules including, for example, cholesterol (¶ 31). "In a certain embodiment, the methods of screening may be used to identify compounds that effect an alteration in late endosomal/lysosomal cholesterol trafficking and thereby reduce or inhibit melanogenesis and/or pigmentation" (¶ 156). Specific compounds known to alter or inhibit late endosomal/lysosomal trafficking are further discussed in paragraphs 176-205.

Examples 5-8 provide experimental results illustrating the connection between pigmentation and an alteration in late endosomal/lysosomal trafficking. These Examples clearly set forth methods by which one of ordinary skill in the art can determine how compounds that affect late endosomal/lysosomal trafficking impact melanogenesis, and how they affect localization of molecules such as tyrosinase. In particular, Example 5 provides a method for detecting the localization of lysosomal hydrolases in a cell, and determining if they are properly targeted to the lysosome (¶¶ 310-320). Example 6 describes a method for determining the effects of compounds that alter late endosomal/lysosomal trafficking on melanin production, and illustrates that such compounds decrease melanin production (¶¶ 321-331). Example 7 illustrates that exposing cells to U18666A (a compound known to alter late endosomal/lysosomal trafficking) does not alter tyrosinase activity, showing that the decreased melanin production associated with U18666A is caused by mislocalization, not by inhibition or alteration of tyrosinase (¶¶ 332-336). Example 8 shows that exposing cells to U18666A alters the

intracellular localization of tyrosinase, thus reinforcing the conclusions drawn in Example 7 (¶¶ 337-341).

In view of the above-referenced disclosure and experimental results in the specification, one of ordinary skill in the art would understand how to practice the methods of claim 47 and 60 and their dependent claims 48-49, 51-56, 61-62, and 64-69 without undue experimentation. Accordingly, Applicants respectfully submit that the present § 112 rejection should be reconsidered and withdrawn.

## II. Rejections Under 35 U.S.C. § 112, Second Paragraph, Indefiniteness

Claims 47-49, 51-56, 60-62, and 64-69 were rejected under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite.

### A. Claims 47-49, 51-56, 60-62, and 64-69

Claims 47-49, 51-56, 60-62, and 64-69 were rejected under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite for recitation of the term "effects an alteration in late endosomal/lysosomal trafficking." Applicants respectfully traverse this rejection.

The term "late endosomal/lysosomal trafficking" is defined in paragraph 220 of the specification:

> The term "late endosomal/lysosomal trafficking" is used herein to refer to the movement of proteins, lipids, or other compounds between different cellular compartments. These locations include the movement of such compounds from the late endosome to the lysosome, from the lysosome to the late endosome, from the late endosome or lysosome to the trans Golgi network, and from the trans Golgi network to the late endosome or lysosome.

An "alteration in late endosomal/lysosomal trafficking" is thus a change in such movements.

The Office Action also expressed some confusion regarding the meaning of "late" with regard to endosomal/lysosomal trafficking. The term "late endosome" refers to a portion of the endosomal compartment found near the Golgi apparatus and the nucleus (*see* Bruce Alberts, et al., Molecular Biology of the Cell 622-625 (3rd ed. 1994)) (Attached hereto as Exhibit A). "Late endosome" is thus a term known to those of ordinary skill in the art.

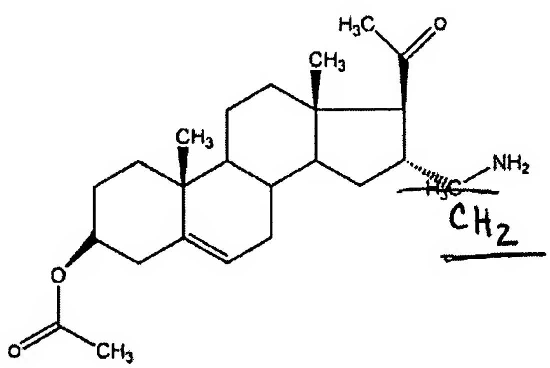
Based on this disclosure, Applicants respectfully submit that one of ordinary skill in the art would understand what is meant by "effects an alteration in late endosomal/lysosomal trafficking."

Accordingly, Applicants respectfully submit that claims 47 and 60, and dependent claims 48-49, 51-54, 56, 61-62, 64-67, and 69 are clear and definite, and thus the present § 112 rejection should be reconsidered and withdrawn.

**B. Claims 56 and 69**

Claims 56 and 69 were rejected under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite because they "recite compounds of formula (III) that contain a carbon atom with five bonds."

Claims 56 and 69 have been amended to recite formula (III) as:



(III)

Accordingly, Applicants respectfully submit that as claims 56 and 59 are clear and definite, the present § 112 rejection should be reconsidered and withdrawn.

## III. Rejections Under 35 U.S.C. § 102(b):

Claims 47-49, 51-52, 55, 60-62, 64-65, 68 were rejected under 35 U.S.C. § 102(b) as allegedly being anticipated by the prior art.

**A. Claims 47, 55, 60, and 68**

Claims 47, 55, 60, and 68 were rejected under 35 U.S.C. § 102(b) as allegedly being anticipated by U.S. Patent No. 5,616,332 to Herstein, which discloses the topical use of sphingosine. Applicants respectfully traverse this rejection.

Applicants' claims 47 and 60 are directed to methods of decreasing melanin production, or of reducing skin pigmentation, by contacting a melanocyte or skin with an effective amount of

a compound that effects an alteration in late endosomal/lysosomal trafficking, including the compound sphingosine.

US 5,616,332 discloses the use of compositions containing sphingosine (col. 6, lines 36-39) to control "long-term irritation produced by repeated applications of small dosages of skin-renewal stimulating acids" (col. 2, lines 21-23).

To anticipate a claim, a reference must teach every element of the claim. MPEP § 2131. US 5,616,332 does not disclose a method of decreasing melanin production or reducing skin pigmentation with a compound that effects an alteration in late endosomal/lysosomal trafficking as claimed. However, the Office Action argues that US 5,616,332 inherently anticipates Applicants' claimed methods.

Inherency requires that the alleged inherent characteristic is <u>necessarily</u> present in the prior art. MPEP § 2112; *see also In re Robertson*, 169 F.3d 743, 745, 49 U.S.P.Q.2d 1949, 1950-51 (Fed. Cir. 1999) ("'Inherency, however, may not be established by probabilities or possibilities. The mere fact that a certain thing may result from a given set of circumstances is not sufficient.'"). Applicants respectfully submit that the compositions disclosed in US 5,616,332 would not <u>necessarily</u> result in carrying out Applicants' claimed methods.

The concentrations of sphingosine disclosed in US 5,616,332 may not be pharmaceutically effective amounts that effect an alteration in late endosomal/lysosomal trafficking and thus decrease melanogenesis as claimed. Although US 5,616,332 teaches that the disclosed compositions may affect age spots, this effect on age spots was not attributed to the sphingosine component. To the contrary, sphingosine is disclosed as being included to reduce irritation from *other* components in the composition (col. 2, lines 21-23; col. 4, lines 50-57). Moreover, the effect on age spots apparently varies based on the pH and proportion of <u>skin-renewal stimulating acid</u> applied to the age spot, <u>not</u> based on the amount of <u>sphingosine</u> (col. 7, lines 1-22; col. 18, line 65 to col. 19, line 2). Thus the sphingosine in the compositions disclosed in US 5,616,332 does not necessarily provide an effective amount to reduce melanin production or skin pigmentation as claimed.

There are many known methods using compositions that do not effect an alteration in late endosomal/lysosomal trafficking, and yet control or inhibit melanin production or skin pigmentation by various different mechanisms, for example, by bleaching existing melanin or

pigment, or preventing new melanin or pigment synthesis by inhibiting the activity of tyrosinase (*see* ¶¶ 18-19). In contrast, Applicants' claims 47 and 60 specifically recite methods using compositions that reduce melanin production or skin pigmentation by the previously unknown mechanism of effecting an alteration in late endosomal/lysosomal trafficking. Because there are so many other possible mechanisms for affecting melanin production or skin pigmentation, and because many methods using compounds that affect melanin production or skin pigmentation do not alter late endosomal/lysosomal trafficking, prior art methods using compositions that reduce melanin production or skin pigmentation by other or unknown mechanisms, such as those disclosed in US 5,616,332, would not inherently possess the characteristics of Applicants' claimed methods.

For these reasons, US 5,616,332 does not anticipate claims 47 and 60, or dependent claims 55 and 68.

Accordingly, Applicants respectfully submit that the rejection of claims 47, 55, 60, and 68 under 35 U.S.C. § 102(b) should be reconsidered and withdrawn.

**B. Claims 47, 49, 51-52, 60, 62, and 64-65**

Claims 47, 49, 51-52, 60, 62, and 64-65 were rejected under 35 U.S.C. § 102(b) as allegedly being anticipated by U.S. Patent No. 5,569,678 to Lee. Applicants respectfully traverse this rejection.

Applicants' claims 47 and 60 are directed to methods of decreasing melanin production, or of reducing skin pigmentation, by contacting a melanocyte or skin with an effective amount of a compound that effects an alteration in late endosomal/lysosomal trafficking, including phenothiazine.

US 5,569,678 discloses the use of phenothiazines, such as trifluoperazine (col. 3, lines 48-49), to soften scar tissue and "to control wound scar production" (col. 4, lines 6-7) via administration "to a wound site for a period of time sufficient to minimize the scar, or to prevent the formation of a hypertrophic scar" (col. 2, lines 21-22).

To anticipate a claim, a reference must teach every element of the claim. MPEP § 2131. US 5,569,678 does not disclose a method of decreasing melanin production or reducing skin pigmentation with a pharmaceutically effective amount of a compound that effects an alteration

in late endosomal/lysosomal trafficking. However, the Office Action argued that US 5,569,678 inherently anticipates Applicants' claimed methods.

As noted above, inherency requires that the alleged inherent characteristic is necessarily present in the prior art. Applicants respectfully submit that practicing the methods disclosed in US 5,569,678 would not necessarily result in carrying out Applicants' claimed methods.

US 5,569,678 describes applying trifluoperazine to wounds or scars, and states that wounds and scar tissue are distinct from skin (*see, e.g.*, col. 1 lines 15-38; col. 4 lines 22-25). Thus the method disclosed in US 5,569,678 certainly would not necessarily decrease melanin production in melanocytes or reduce skin pigmentation as claimed.

Thus US 5,569,678 does not expressly or inherently anticipate the methods of claims 47 or 60, or dependent claims 49, 51-52, 62, and 64-65.

Accordingly, Applicants respectfully submit that the present rejection under 35 U.S.C. § 102(b) should be reconsidered and withdrawn.

**C. Claims 47-48 and 60-61**

Claims 47-48 and 60-61 were rejected under 35 U.S.C. § 102(b) as allegedly being anticipated by U.S. Patent No. 4,439,432 to Peat. Applicants respectfully traverse this rejection.

Applicants' claims 47 and 60 are directed to methods of decreasing melanin production, or of reducing skin pigmentation, by contacting a melanocyte or skin with an effective amount of a compound that effects an alteration in late endosomal/lysosomal trafficking, including the compound progesterone.

US 4,439,432 describes a progesterone composition that may be used to correct progesterone deficiency (col. 1, lines 11-12), or for "treatment of psoriasis, eczema, senile skin changes including warts, superficial burns, allergies, abnormal hair growth resulting from androgen excess, intestinal inflammation and bowel spasms, migraine, and for promoting maturation of thymus-derived lymphocytes, and as a contraceptive" (col. 1, lines 51-56). US 4,439,432 does not disclose a method of decreasing melanin production or reducing skin pigmentation with a compound that effects an alteration in late endosomal/lysosomal trafficking. However, the Office Action argues that US 4,439,432 inherently anticipates Applicants' claimed methods.

Applicants respectfully submit that the methods and compositions disclosed in US 4,439,432 do not necessarily possess the characteristics of Applicants' claimed methods. US 4,439,432 gives no indication that the progesterone in the disclosed composition is present in pharmaceutically effective amounts or properly formulated to effect an alteration in late endosomal/lysosomal trafficking, and thus reduce melanin production or skin pigmentation.

Thus Peat does not expressly or inherently anticipate the methods of claims 47 or 60, or dependent claims 48 and 61.

Accordingly, Applicants respectfully submit that the present rejection under 35 U.S.C. § 102(b) should be reconsidered and withdrawn.

**D. Claims 47 and 60**

Claims 47 and 60 were rejected under 35 U.S.C. § 102(b) as allegedly being anticipated by Hashizume et al., CA 126:190762, which discloses the use of pregnenolones as melanin formation inhibitors.

Without acquiescing in the propriety of this rejection, in order to expedite prosecution, Applicants have amended claims 47 and 60 to replace generic Formula I with the specific compounds of Formulae (II) through (VIII). CA 126:190762 does not disclose Formulae II-VIII, therefore it does not expressly or inherently anticipate the methods of claims 47 or 60.

Accordingly, Applicants respectfully submit that the present rejection under 35 U.S.C. § 102(b) should be reconsidered and withdrawn.

## CONCLUSION

In view of the amendments and arguments set forth above, Applicants respectfully submit that the rejections contained in the Office Action of January 22, 2007 have been overcome, and that the pending claims are in condition for allowance.

No fees are believed to be due in connection with this correspondence. However, please charge any required fees or credit any overpayments to our Deposit Account No. 08-0219.

The Examiner is encouraged to telephone the undersigned at the number listed below in order to expedite the prosecution of this application.

Respectfully submitted,

Dated: April 18, 2007

Ann-Louise Kerner
Ann-Louise Kerner, Ph.D.
Reg. No. 33,523

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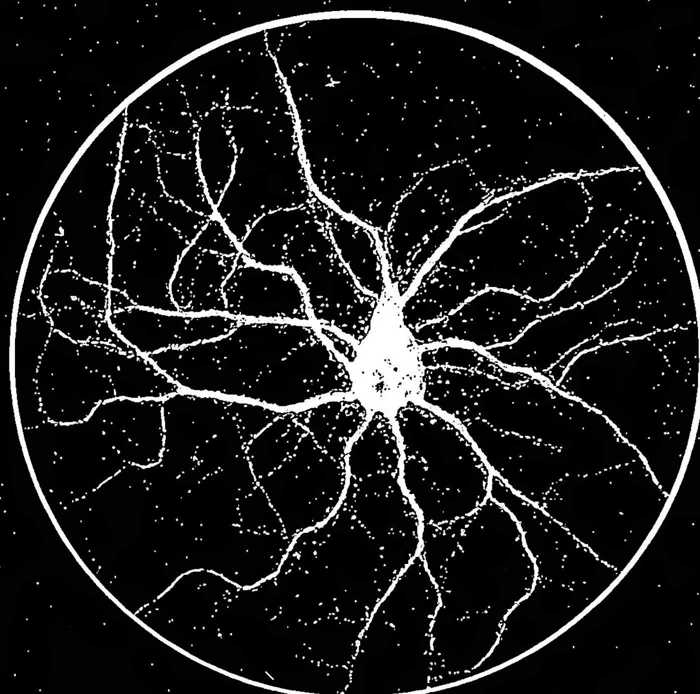
# MOLECULAR BIOLOGY OF THE CELL

THIRD EDITION



Bruce Alberts • Dennis Bray
Julian Lewis • Martin Raff
Keith Roberts • James D. Watson

Exhibit A

# MOLECULAR BIOLOGY OF
# THE CELL
## THIRD EDITION

Bruce Alberts • Dennis Bray
Julian Lewis • Martin Raff • Keith Roberts
James D. Watson

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**Front cover:** The photograph shows a rat nerve cell in culture. It is labeled with a fluorescent antibody that stains its cell body and dendritic processes (*yellow*). Nerve terminals (*green*) from other neurons (not visible), which have made synapses on the cell, are labeled with a different antibody. (Courtesy of Olaf Mundigl and Pietro de Camilli.)

**Dedication page:** Gavin Borden, late president of Garland Publishing, weathered in during his mid-1980s climb near Mount McKinley with MBoC author Bruce Alberts and famous mountaineer guide Mugs Stump (1940–1992).

**Back cover:** The authors, in alphabetical order, crossing Abbey Road in London on their way to lunch. Much of this third edition was written in a house just around the corner. (Photograph by Richard Olivier.)

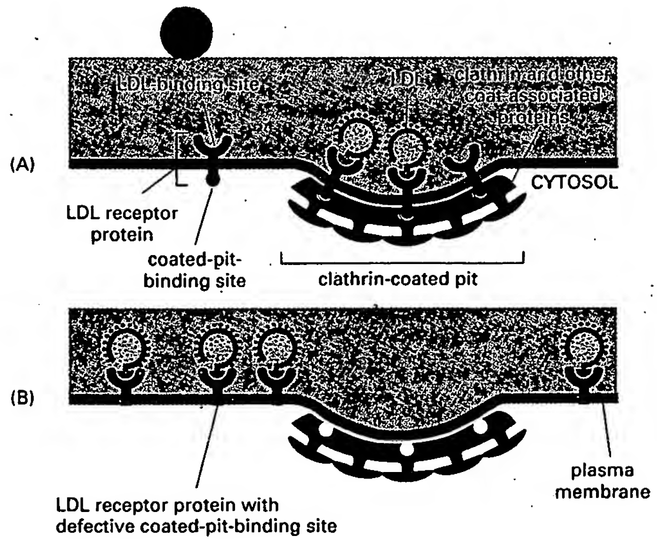



**Figure 13–30 Normal and mutant LDL receptors.** (A) LDL receptor proteins binding to a coated pit in the plasma membrane of a normal cell. The human LDL receptor is a single-pass transmembrane glycoprotein composed of about 840 amino acid residues, only 50 of which are on the cytoplasmic side of the membrane. (B) A mutant cell in which the LDL receptor proteins are abnormal and lack the site in the cytoplasmic domain that enables them to bind to coated pits. Such cells bind LDL but cannot ingest it. In most human populations 1 in 500 individuals inherits one defective LDL receptor gene and, as a result, is likely to die prematurely from a heart attack caused by atherosclerosis.

receptor, enter coated pits irrespective of whether they have bound their specific ligands; others enter only with a specific ligand bound, suggesting that a ligand-induced conformational change is required for them to bind to the pits. Since most plasma membrane proteins fail to accumulate in clathrin-coated pits, the pits must function as molecular filters, collecting certain plasma membrane proteins (receptors) and excluding others. Electron microscopic studies of cultured cells exposed to different ligands (labeled to make them visible in the electron microscope) have demonstrated that many kinds of receptors cluster in the same coated pit. The plasma membrane of one clathrin-coated pit can probably accommodate about 1000 receptors of assorted varieties. Although all of the receptor-ligand complexes that utilize this endocytic pathway apparently are delivered to the same endosomal compartment, the subsequent fates of the endocytosed molecules vary, as we now discuss.

## Endocytosed Materials Often End Up in Lysosomes [24]

The **endosomal compartment** can be made visible in the electron microscope by adding a readily detectable tracer molecule, such as the enzyme peroxidase, to the extracellular medium and leaving the cells for varying lengths of time to take it up by endocytosis. The distribution of the molecule after its uptake reveals the endosomal compartment as a complex set of heterogeneous membrane-bounded tubes and vesicles extending from the periphery of the cell to the perinuclear region, where it is often close to the Golgi apparatus, although they are clearly distinct. Two sets of endosomes can be readily distinguished in such labeling experiments: the tracer molecule appears in **early endosomes,** just beneath the plasma membrane, within a minute or so and in **late endosomes,** close to the Golgi apparatus and near the nucleus, after 5 to 15 minutes (Figure 13–31).

As mentioned earlier, the interior of the endosomal compartment is kept acidic (pH ~6) by ATP-driven $H^+$ pumps in the endosomal membrane that pump $H^+$ into the lumen from the cytosol; in general, late endosomes are more acidic than early endosomes. This acidic environment plays a crucial part in the function of these organelles. A similar or identical *vacuolar $H^+$ ATPase* is thought to acidify all endocytic and exocytic organelles, including phagosomes, lysosomes, selected compartments of the Golgi apparatus, and many transport and secretory vesicles.

We have already seen how endocytosed materials that reach the late endosomes become mixed with newly synthesized acid hydrolases and end up in lysosomes. Many molecules, however, are specifically diverted from this journey to destruction and are recycled instead from the early endosomes back to the

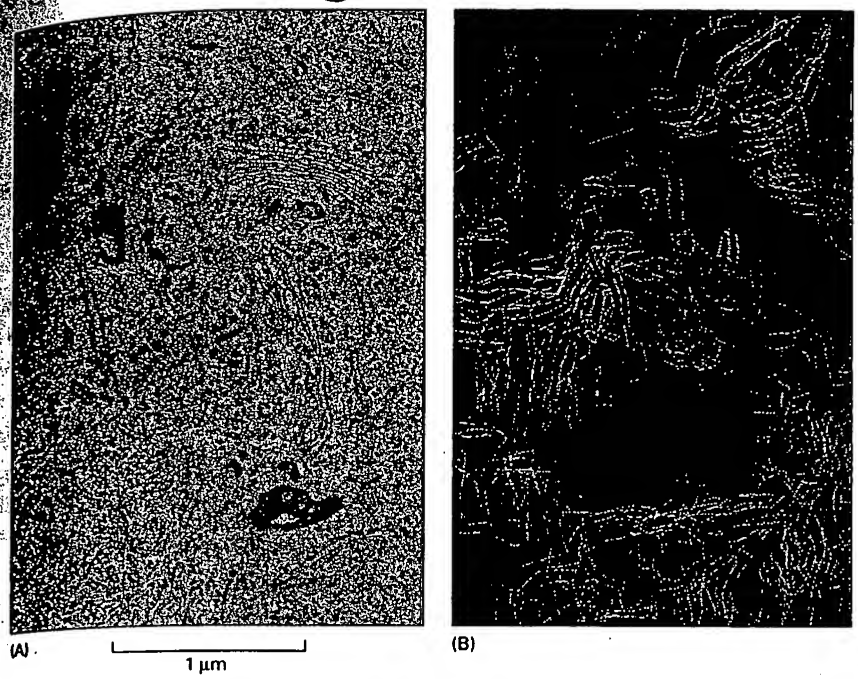



**Figure 13–31 The relationship of late endosomes to other membrane-bounded compartments.** (A) Baby hamster kidney (BHK) cells in culture were incubated in a solution containing the enzyme peroxidase for 15 minutes, which was long enough for the peroxidase to be taken up by fluid-phase endocytosis and delivered to late endosomes but not long enough for it to be delivered to lysosomes. After the cells were fixed and exposed to a peroxidase substrate, the product of the enzymatic reaction was made electron dense by fixation with osmium tetroxide. (B) Serial reconstructions of late endosomes (*blue*), ER (*yellow*), and Golgi apparatus (*red*) prepared from electron micrographs, one of which is shown in (A). The reconstruction was drawn from 18 serial thin sections. The nucleus is indicated by N in (A) and is shown in *green* in (B). (A, from M. Marsh, G. Griffiths, G. Dean, I. Mellman, and A. Helenius, *Proc. Natl. Acad. Sci. USA* 83:2899–2903, 1986; B, courtesy of Mark Marsh.)

plasma membrane via transport vesicles. Only those molecules that are not retrieved from endosomes are degraded.

## Specific Proteins Are Removed from Early Endosomes and Returned to the Plasma Membrane [25]

The early endosomal compartment acts as the main sorting station in the endocytic pathway, just as the *trans* Golgi network serves this function in the biosynthetic-secretory pathway. In the acidic environment of the early endosome, many internalized receptor proteins change their conformation and release their ligand, just as the M6P receptors unload their cargo of acid hydrolases in the even more acidic late endosomes. Those endocytosed ligands that dissociate from their receptors in the early endosome are usually doomed to destruction in lysosomes, along with the other non-membrane-bound contents of the endosome. Some other endocytosed ligands, however, remain bound to, and thereby share the fate of, their receptors.

The fates of the receptor proteins—and of any ligands remaining bound to them—vary according to the specific type of receptor. (1) Most receptors return to the same plasma membrane domain from which they came; (2) some receptors progress to lysosomes, where they are degraded; and (3) some receptors proceed to a different domain of the plasma membrane, thereby mediating a process called *transcytosis* (Figure 13–32).

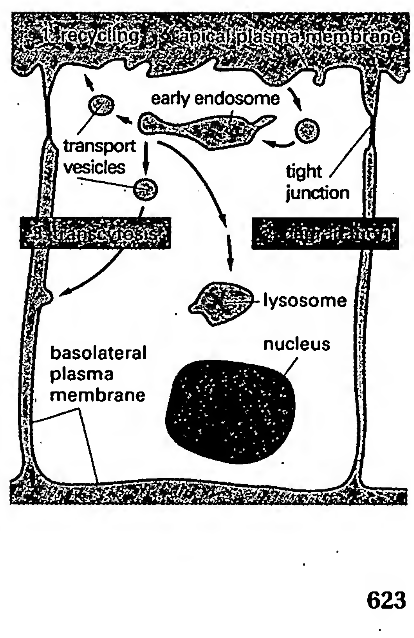



**Figure 13–32 Possible fates for transmembrane receptor proteins that have been endocytosed.** Three pathways from the endosomal compartment in an epithelial cell are shown. Receptors that are not specifically retrieved from endosomes follow the pathway from the endosomal compartment to lysosomes, where they are degraded. Retrieved receptors are returned either to the same plasma membrane domain from which they came (*recycling*) or to a different domain of the plasma membrane (*transcytosis*). If the ligand that is endocytosed with its receptor stays bound to the receptor in the acidic environment of the endosome, it will follow the same pathway as the receptor; otherwise it will be delivered to lysosomes.

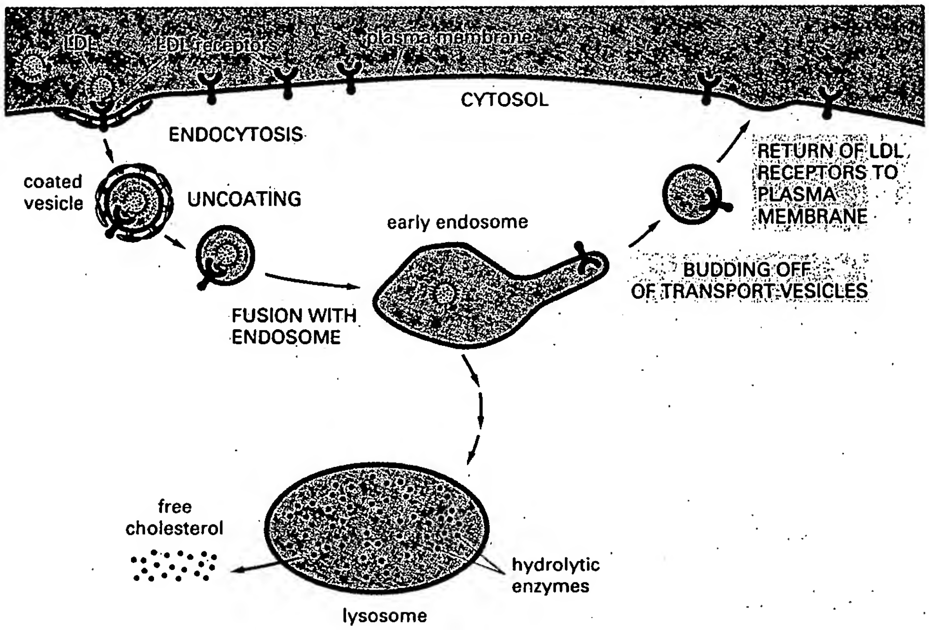



**Figure 13–33 Receptor-mediated endocytosis of LDL.** Note that the LDL dissociates from its receptors in the acidic environment of the endosome. After a number of steps (see Figure 13–34) the LDL ends up in lysosomes, where it is degraded to release free cholesterol. In contrast, the LDL receptor proteins are returned to the plasma membrane via transport vesicles that bud off from the tubular region of the endosome, as shown. For simplicity, only one LDL receptor is shown entering the cell and returning to the plasma membrane. Whether it is occupied or not, an LDL receptor typically makes one round trip into the cell and back to the plasma membrane every 10 minutes, making a total of several hundred trips in its 20-hour life-span.

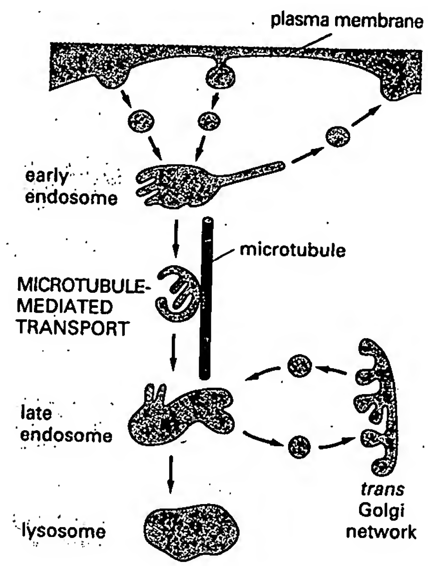
The LDL receptor follows the first pathway. It dissociates from its ligand LDL in the endosome and is recycled to the plasma membrane for reuse, while the discharged LDL is carried to lysosomes (Figure 13–33). A similar but more complex recycling occurs following the endocytosis of **transferrin,** a protein that carries iron in the blood. Cell-surface transferrin receptors deliver transferrin with its bound iron to early endosomes by receptor-mediated endocytosis. The low pH in the endosome induces transferrin to release its bound iron, but the iron-free transferrin itself (called apotransferrin) remains bound to its receptor and is recycled back to the plasma membrane as a receptor-apotransferrin complex. When it has returned to the neutral pH of the extracellular fluid, the apotransferrin dissociates from the receptor and is thereby freed to pick up more iron and begin the cycle again. Thus the transferrin protein shuttles back and forth between the extracellular fluid and the endosomal compartment, avoiding lysosomes and delivering the iron that cells need to grow.

The second pathway that endocytosed receptors can follow from endosomes is taken by the receptor that binds *epidermal growth factor (EGF),* a small protein that stimulates epidermal and various other cells to divide. Unlike LDL receptors, these receptors accumulate in coated pits only after binding EGF. Moreover, most of them do not recycle but end up in lysosomes, where they are degraded along with the ingested EGF. EGF binding therefore leads to a decrease in the concentration of EGF receptors on the cell surface—a process called *receptor down-regulation.* As a result, the concentration of signaling ligand in the extracellular fluid regulates the number of its complementary receptor molecules on the target-cell surface (discussed in Chapter 15).

## The Relationship Between Early and Late Endosomes Is Uncertain [26]

It is unclear how endocytosed molecules move from one endosomal compartment to another so as to end up in lysosomes. One view is that early endosomes slowly move inward to become late endosomes, which, as a result of fusion with hydrolase-bearing transport vesicles from the *trans* Golgi network, continuous membrane retrieval, and increasing acidification, are converted to lysosomes. Another view is that early and late endosomes are separate stationary compartments and that transport between them occurs via an intermediate transport compartment—either by a dynamic network of tubes or by the pinching off of pieces of the early endosome that are transported to the cell interior, where they

**Figure 13–34 The endocytic pathway from the plasma membrane to lysosomes.** Transport from early to the late endosome is mediated by large *endosomal carrier vesicles,* which contain large amounts of invaginated membrane and are therefore called *multivesicular bodies.* It is uncertain whether they should be regarded as middle-aged endosomes moving toward the cell interior as they mature or as distinct transport compartments. The movement occurs along microtubules and can be experimentally blocked with microtubule-depolymerizing drugs. Eventually, the late endosome is thought to convert into a lysosome. Transport vesicles recycle material between the early endosome and the cell surface, while a different set of transport vesicles recycle material between the late endosome and the *trans* Golgi network.




eventually fuse with late endosomes (Figure 13–34). Early and late endosomes do, in fact, differ in their protein composition: in particular, they are associated with different rab proteins, which play an important part in directing vesicular transport, as we discuss later (see Table 13–1, p. 644).

## Macromolecules Can Be Transferred Across Epithelial Cell Sheets by Transcytosis [27]

Some receptors on the surface of polarized epithelial cells transfer specific macromolecules from one extracellular space to another by a process called **transcytosis.** These receptors follow the third pathway from endosomes (see Figure 13–32). A newborn rat, for example, obtains antibodies from its mother's milk (which help protect it against infection) by transporting them across the epithelium of its gut. The lumen of the gut is acidic, and at this low pH the antibodies in the milk bind to specific receptors on the apical (absorptive) surface of the gut epithelial cells and are internalized via clathrin-coated pits and vesicles and are delivered to early endosomes. The receptor-antibody complexes remain intact and are retrieved in transport vesicles that bud from the early endosome and subsequently fuse with the basolateral domain of the plasma membrane. On exposure to the neutral pH of the extracellular fluid that bathes the basolateral surface of the cells, the antibodies dissociate from their receptors and eventually enter the newborn's bloodstream. The secretion of these antibodies into the mother rat's milk also occurs by transcytosis, but in the reverse direction, from blood to milk. Other mammals, including humans, also transport antibodies into milk in this way, but the antibodies remain in the infant's gut and do not, as in the rat, enter the bloodstream.

The variety of pathways that different receptors follow from endosomes implies that, in addition to binding sites for their ligands and binding sites for coated pits, many receptors also possess sorting signals that guide them into the appropriate type of transport vesicle leaving the endosome and thereby to the appropriate target membrane in the cell.

## Epithelial Cells Have Two Distinct Early Endosomal Compartments But a Common Late Endosomal Compartment [28]

In polarized epithelial cells, endocytosis occurs from both the basolateral and the apical domains of the plasma membrane. Material endocytosed from either domain first enters an early endosomal compartment that is unique to that domain. This arrangement allows endocytosed receptors to be recycled back to their original membrane domain, unless they contain signals that mark them for transcytosis to the other domain. Molecules endocytosed from either domain that are not retrieved from the early endosomes are transported to a common late endosomal compartment near the cell center and are eventually degraded in lysosomes (Figure 13–35).